

Nicotine metabolite ratio as a predictor of cigarette consumption

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The rate of nicotine metabolism is hypothesized to be a determinant of how much a person smokes. That is, rapid metabolizers would be expected to need more nicotine and, therefore, smoke more than slow metabolizers. Nicotine is metabolized extensively by the liver enzyme CYP2A6, primarily to cotinine. Cotinine is itself metabolized by CYP2A6 to 3'-hydroxycotinine (3-HC). The ratio of metabolite to parent (i.e., 3-HC:cotinine) would be expected to reflect CYP2A6 activity. We measured the ratio of 3-HC:cotinine in the urine of 72 smokers. This ratio was significantly correlated with the number of cigarettes smoked per day ($r=.33$, $p=.005$), though not with the Fagerström Test for Nicotine Dependence. This finding supports the hypothesis that the rate of nicotine metabolism is a determinant of the level of cigarette consumption and supports the use of the 3-HC:cotinine ratio as a noninvasive marker of nicotine metabolism.

Introduction

It has been suggested that the rate of nicotine metabolism is a determinant of nicotine intake from cigarettes (Benowitz, 1999b; Pianezza, Sellers, & Tyndale, 1998). Nicotine is metabolized primarily by the liver enzyme CYP2A6 (Messina, Tyndale, & Sellers, 1997; Nakajima et al., 1996b). Cotinine, the primary metabolite of nicotine, also is metabolized by CYP2A6, predominantly to the metabolite 3'-hydroxycotinine (3-HC; Nakajima et al., 1996a). The ratio of the enzyme product, 3-HC, to the precursor, cotinine, would be expected to reflect CYP2A6 activity and could serve as a marker for nicotine metabolism rate.

Evidence suggests that people smoke cigarettes to achieve particular levels of nicotine in the body that produce desired psychopharmacological effects

(Benowitz, 1999b). Thus, faster metabolism of nicotine (i.e., greater CYP2A6 activity) could lead to the need to smoke more cigarettes in order to maintain a desired level of nicotine in the body. This would result in a greater intake of tobacco smoke toxins and a potentially higher risk of tobacco-related diseases. CYP2A6 activity also is linked to the bioactivation of tobacco-specific nitrosamines, which are potent carcinogens that contribute to smoking-related cancer – another potential link between higher CYP2A6 activity and a greater risk of smoking-related cancer (Pelkonen & Raunio, 1995; Yamazaki, Inui, Yun, Guengerich, & Shimada, 1992).

The most accurate method for determining nicotine metabolic rate is to give an intravenous infusion of nicotine, measure blood levels, and determine the clearance (Benowitz & Jacob, 1994). This approach, however, is not feasible for population studies. A noninvasive method for estimating the rate of nicotine metabolism in larger numbers of people would be useful as a phenotypic marker of CYP2A6 activity and, therefore, of the rate of nicotine metabolism. Cotinine is metabolized to 3-HC by the same enzyme responsible for the metabolism of nicotine. Because cotinine has a relatively long half-life (averaging 16 hr), cotinine levels are fairly stable in the blood and urine of smokers over time (Benowitz, 1999a). 3'-Hydroxycotinine is eliminated more rapidly than

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Table 1. Demographics and nicotine metabolite excretion data (mean \pm SD)

	All participants (<i>n</i> = 72)	White (<i>n</i> = 61)	Others (<i>n</i> = 11)	Significance (<i>p</i> value)
Age (years)	37.4 \pm 12.4	37.3 \pm 12.9	37.7 \pm 9.3	NS
Female (%)	56.9	59.0	45.5	NS
Body mass index	25.9 \pm 5.9	25 \pm 4.8	31.6 \pm 9	.046
Education (years)	13.8 \pm 2.1	13.8 \pm 2.2	13.2 \pm 1.5	NS
Smoking rate (cigarettes/day)	21.8 \pm 8.9	22.5 \pm 8.8	17.7 \pm 9.2	NS
Fagerström Tolerance Questionnaire score	6.7 \pm 2.2	6.8 \pm 2.1	6.2 \pm 2.6	NS
Fagerström Test for Nicotine Dependence score	5.7 \pm 2.2	5.7 \pm 2.2	5.5 \pm 2.4	NS
Urine cotinine (ng/ml) ^a	1,451 \pm 868	1,526 \pm 899	1,038 \pm 527	NS
Urine 3'-hydroxycotinine (3-HC) (ng/ml) ^a	8,001 \pm 8,616	8,877 \pm 8,959	3,146 \pm 3,859	NS
Urine 3-HC:cotinine ratio	5.2 \pm 3.9	5.5 \pm 3.8	3.4 \pm 4.2	.09

NS, not significant.

^aTotal of unconjugated and conjugated metabolite.

cotinine (Benowitz & Jacob, 2001), but because it is constantly formed from cotinine, the time course of 3-HC would be expected to parallel that of cotinine. Because the curves are parallel, the ratio of 3-HC:cotinine should be stable over time. This ratio (a reflection of the degree of separation of the cotinine and 3-HC curves over time) might then be a convenient noninvasive marker of CYP2A6 activity.

Method

Subjects

A total of 72 smokers (57% female) were recruited from the local community to participate in an assessment of a test strip for detecting nicotine and metabolites in urine. Of the recruited smokers, 61 (85%) identified themselves as White, 10 were Black, and one was Asian.

Participant characteristics for White vs. other races combined are shown in Table 1.

Procedure

Information provided by participants included demographic data, self-reported smoking rate (cigarettes per day), and Fagerström Test for Nicotine Dependence (FTND) scores (Fagerström & Schneider, 1989). Subjects provided a urine sample at the time of interview.

Urinary cotinine and the sum of unconjugated and conjugated 3-HC were measured via gas chromatography as described previously (Benowitz, Jacob, Fong, & Gupta, 1994).

Data analysis

Data were expressed as the sum of unconjugated and conjugated cotinine and 3-HC. Both cotinine and 3-HC are metabolized in part by glucuronidation (Benowitz et al., 1994). The glucuronidation pathway is not mediated by CYP2A6. The best measures of

total cotinine exposure and of 3-HC generation are expected to be the sum of unconjugated plus conjugated forms. We computed the ratio as a potential marker of CYP2A6 activity: Total 3-HC divided by total cotinine. Using linear regression analysis, we examined the ratio as a predictor of cigarette consumption and of FTND score. Results for Whites vs. Blacks and Asians were compared because of known ethnic differences in cotinine metabolism among these three ethnic groups (Benowitz et al., 1999; Benowitz, Perez-Stable, Herrera, & Jacob, 2000). Whites metabolize nicotine and cotinine more rapidly than do Blacks or Asians. The ethnic groups were compared by *t* tests on log-transformed data.

Results

The average urine concentrations of total (unconjugated plus conjugated) cotinine and 3-HC are shown in Table 1. The distribution of urine 3-HC:cotinine ratios in the study sample is shown in Figure 1. The 3-HC:cotinine ratio was correlated significantly with the number of cigarettes smoked per day ($r = .33$, $p = .005$) (Figure 2). The 3-HC:cotinine ratio tended to be higher in Whites compared with other races

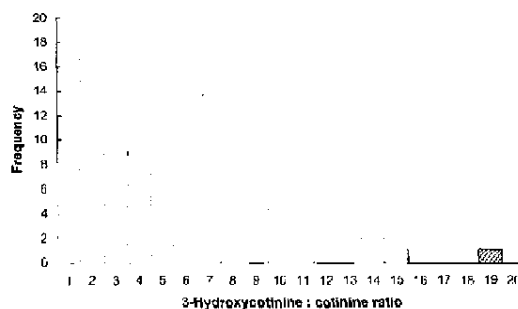


Figure 1. Frequency histogram showing the distribution of urine 3'-hydroxycotinine:cotinine ratios in the study sample.

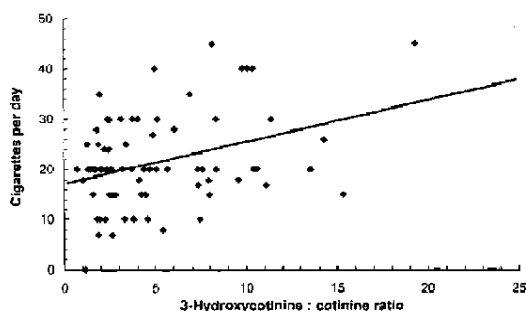


Figure 2. Correlation between urine ratios of 3'-hydroxycotinine:cotinine and the number of cigarettes smoked per day. The correlation coefficient, $r = .33$, $p = .005$.

($p = .09$). No significant correlation was found between the metabolite ratio and nicotine dependence as measured by the FTND. Scores on the FTND were correlated significantly with cigarettes per day ($r = .78$; $p < .001$), expired carbon monoxide ($r = .48$, $p < .001$), urine cotinine ($r = .28$, $p = .02$), and urine 3-HC ($r = .26$, $p = .04$).

Discussion

We propose that the ratio of 3-HC:cotinine reflects the rate of metabolism of cotinine to 3-HC, a marker of CYP2A6 activity in smokers. CYP2A6 is the primary enzyme responsible for nicotine metabolism (Messina et al., 1997; Nakajima et al., 1996b). Research has demonstrated a significant inverse correlation between plasma 3-HC:cotinine ratio and plasma nicotine in smokers during *ad libitum* smoking (Benowitz & Jacob, 2001). That finding is consistent with the idea that the higher the ratio of 3-HC:cotinine, the greater the CYP2A6 activity, and the faster the clearance of nicotine. Assuming that regulation of nicotine intake is not perfect, the faster clearance of nicotine would predict a lower plasma nicotine concentration for a given intake of nicotine.

The present study extended that observation, finding a significant positive correlation between urine 3-HC:cotinine ratio and cigarette consumption. This finding supports the hypothesis that the rate of nicotine metabolism is associated with the level of cigarette consumption.

One conceivable confounder of the interpretation of our findings that faster nicotine metabolism leads to smoking more cigarettes is that heavier smoking results in higher 3-HC:cotinine ratios because of induction of nicotine metabolism. This appears not to be the case based on prior research showing that smoking inhibits rather than accelerates nicotine metabolism (Benowitz & Jacob, 2000).

We did not find a significant relationship between the metabolite ratio and the level of dependence, as determined by the FTND. Once an individual becomes a regular smoker, the rate of nicotine metabolism might not be important. That is, smokers might adjust their level of smoking to obtain a desired level of nicotine, smoking more or less depending on their rate of nicotine metabolism, independent of level of dependence. Nicotine clearance could have an effect on the transition from experimental to addicted smoking, as suggested by Pianezza et al. (1998). We did not look at transition. Also, FTND score may not be the most appropriate phenotype for dependence that would be sensitive to the rate of nicotine metabolism. More investigation of this issue is warranted.

We found substantial differences in average metabolite ratios between Whites and non-Whites (10 Blacks and 1 Asian) that were marginally significant even in this small sample. The lower value for metabolite ratios in Blacks and Asians compared with Whites is consistent with previous research in which nicotine and cotinine metabolism were studied directly (Benowitz et al., 1999, 2000). This observation is important because the use of 3-HC:cotinine ratios as a biomarker of the rate of nicotine metabolism requires that ethnicity be considered as a covariant.

Acknowledgments

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